Acta Bot. Neerl. 37(2), June 1988, p. 321–325

Meetings of the Royal Botanical Society of The Netherlands

MEETING OF THE SECTION FOR PLANT MORPHOLOGY, ANATOMY AND CYTOLOGY ON 19 JUNE 1987

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REORGANIZATION OF CORTICAL MICROTUBULES IN TOBACCO EXPLANTS

Immunofluorescence studies were carried out on tissue explants of *Nicotiana tabacum* embedded in polyethylene glycol (Hawes *et al.* 1983). The orientation of cortical microtubules with respect to the cell axis gradually changed from transverse to longitudinal during the first 6 h of culturing. These changes might occur by assembly of tubulins at "+"-ends of microtubules in new directions, rather than by reorientation of complete Mt-helices (Roberts *et al.* 1985). Before cell division preprophase bands of microtubules were formed parallel to the plane of the cell wall to be formed. Cells of the protrusions, from where floral buds will develop (Wilms & Sassen 1987), had randomly oriented microtubules. The initiation of protrusion formation occurred between 24 and 48 h after tissue explantation.

Hawes, C., Juniper, B.E. & Horne, J.C. (1983): Electron microscopy of resin-free sections of plant cells. *Protoplasma*, **115:** 88–93.

Roberts, I.N., Lloyd, C.W. & Roberts, K. (1985): Ethylene-induced microtubule reorientation: mediation by helical arrays. *Planta*, **164:** 439–447.

Wilms, F.H.A. & Sassen, M.M.A. (1987): Origin and development of floral buds in tobacco explants. *New Phytologist*, **105:** 57–65.

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A MORPHOMETRIC ANALYSIS OF MAIZE EMBRYO ROOT DEVELOPMENT DURING *IN VITRO* CULTURE

Cell division and cell growth are important factors in tissue differentiation of the embryo root. Rate and direction of these factors determine the ultimate tissue pattern. Cell dimensions of the various tissues of the embryo root, grown *in vitro* during 0–48 h, were analysed by morphometric methods, using a MOP-30 (Zeiss) image analyser. In the roots, from tip to base, several regions were distinguished. Of these regions the average cell length (parallel to the axis) and cell width (perpendicular to the root axis) were measured for the various tissues.

From tip to base, cell length increased in the region just after the tip, while subapically a region was found with many divisions in the transverse direction, which results in an average decrease in length. In some tissues this division zone was oriented always at the same distance from the tip, while in, for example, the cortex this region moved from tip to base during culture. Further away from the root tip most tissues showed a gradual increase in length towards the base, although in some tissues (epidermis, cortex, vascular tissue) many transversal divisions were also observed.

From root tip to root base changes in cell width are different for the various tissues. Tissues with only one cell layer (epidermis, endodermis, pericycle) show a gradual increase in width from tip to base, while the other tissues generally show a decrease in width just after the tip, due to many longitudinal divisions. In the latter, the width increases gradually towards the base.

Clear conclusions about development during culturing time cannot be reached for all regions. While due to *in vitro* culture in the tip region, a simultaneous activation of the quiescent centre is expected and divisions in the various tissues are found at different times. In the central regions a decrease in cell length is found in all tissues, while the division region moves from tip to base over a period of time. At the base a decrease in cell length occurs for most tissues, only in cells of the vascular tissue and pith elongation is going to dominate during time.

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BEGONIA SEEDS AND THEIR ADAPTATIONS TO DISPERSAL

A great diversity in shape and micromorphology of the seeds is present within the family Begoniaceae. This is not only correlated with taxonomic differences but also with dispersal strategies.

Most Begonias are anemochorous; in the anemoballistic species the wind shakes the winged fruits and scatters the minute seeds. Surface roughness of the seeds, caused by a reticulate cell wall pattern and cuticular sculpture, promotes microturbulence and slows down the rate of fall. In several *Begonia* sections the seeds show special adaptations to anemochory. In the African section, *Cristasemen*, and the Brazilian sections, *Enita* and *Solananthera*, the micropylar and chalazal ends are composed of blown, air-filled cells functioning as balloons. Scobiform seeds are found in some Asiatic and American sections. The presence of these seed types is often found in epiphytic and/or lianoid species.

Rain-ballists are found in the Asiatic section *Platycentrum*. The two shorter wings form a cup to catch raindrops. In the section *Casparya*, which inhabits the Andean cloud forest, the fruit wings are replaced by horns and probably act as rattle burrs operated by animals.

The African sections *Baccabegonia, Mezierea, Squamibegonia* and *Tetraphila* have indehiscent or dehiscent fleshy fruits and are supposed to be zoochorous. The relatively large seeds are smooth, without cuticular sculpture and may be provided with an aril. The section *Scutobegonia*, which grows on the floor of the African rain forest, has indehiscent fruits that decay gradually. The small seeds have prominent cuticles and are supposed to be dispersed by rain wash or epizoochory.

We conclude that in the large genus *Begonia*, differences in fruit and seed structure can often be related to habitat and dispersal.

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LOCALIZATION OF EXTRA- AND INTRACELLULAR PEA ROOT LECTIN BY DOUBLE IMMUNOGOLD LABELLING

In order to study the role of pea root lectin during the early infection steps of *Pisum* by *Rhizobium*, we investigated, ultrastructurally, the localization of lectin on and in young root hairs.

As established by immunofluorescence microscopy, lectin is excreted only by a minority of growing root hairs (Diaz *et al.* 1986). To increase the chance of ultrathin sectioning such hairs, we pre-immunolabelled the hairs on whole root pieces using protein A-40 nm gold conjugate. Although the lectin-excreting hairs stood out by their red tips (Goosen-de Roo *et al.* 1987), processing of these hairs for electron microscopy was met with difficulties.

For the pre-labelling we used the method described by Diaz *et al.* (1986), using as a second layer in the immunolabelling of the protein A-40 nm gold conjugate. The pre-labelling appeared to be

destructive to the ultrastructure of the hairs. The root pieces had to be fixed before the labelling. Glutaraldehyde (0·1%), 2% paraformaldehyde and 0·1% acroleine in a 0·1 M sodiumcacodylate buffer at pH 7·4 over 30 min gave reasonable results. After the immunolabelling a post-fixation was necessary to cross-link the specific antibody and the protein A-40 nm gold conjugate. After that the material was dehydrated and infiltrated with Lowicryl K4M or LRwhite resin as embedding medium.

A second difficulty was that the red coloured gold stain at the tip of lectin-excreting root hairs disappeared within 24 h. Therefore, the long lasting procedure of embedding in Lowicryl K4M had to be abandoned. With LRwhite the red coloration of hair tips was preserved. However, the root hairs were burst at their tips, presumably by the too rapid LRwhite procedure.

From these results we must conclude that the proposed pre-selection of lectin-excreting root hairs is not practical. The main cause was probably the high solubility of pea root lectin.

Diaz, C.L., van Spronsen, P.C., Bakhuizen, R., Logman, G.J.J., Lugtenberg, E.J.J. & Kijne, J.W. (1986): Correlation between infection by *Rhizobium leguminosarum* and lectin on the surface of *Pisum sativum* L. roots. *Planta*, **168:** 350–359.

Goosen-de Roo, L., Bominaar, A.A. & Diaz, C.L. (1987): Immunogold staining of surface root lectin in *Pisum sativum* L. using a protein A-40 nm gold complex. *Acta Bot. Neerl.* **36:** 146–147.

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WOOD ANATOMY OF CONNARACEAE IN THE LIGHT OF REDUCTION OF THE NUMBER OF GENERA

The secondary xylem of 55 species from 19 genera of Connaraceae was studied. The main purpose of this investigation was to present wood anatomical arguments for reduction of the number of genera in this family, which is 24. According to several taxonomists this should be done. A general wood anatomical description of the family is given, based on our own investigations and on measurements made by van Veenendaal (1964). Descriptions were also made of the separate genera; Dickison's (1972) measurements in this family were added for comparison and to create a more complete picture of the family. Four groups of genera are composed, mainly based on the composition of the ground tissue; two groups are divided into smaller subgroups. These genera groups are:

1. *Manotes* with *Pseudoconnarus*.
2. *Rourea* with *Byrsocarpus, Paxia* and *Jaundea; Spiropetalum, Santoloidella* and *Santaloides; Bernardinia, Cnestis* and *Cnestidium*. The last two genera possess a wide range of wood anatomical characters.
3. *Agelaea* with *Castanola*.
4. *Connarus* with *Hemandradenia, Ellipanthus*, next to *Jollydora* and *Burttia*.

In the series mentioned above, *Manotes* is the most primitive genus, *Connarus* with *Burttia*, as an extreme, the most advanced. Within each group the genera resemble each other in many aspects. However, the reduction of all group genera to the representative group genus, based on wood anatomical characters only, is not justified. On the other hand, support can be found in wood characters to place some genera in synonymy under the representative one; also genera of the subgroup with *Spiropetalum, Santaloidella* and *Santaloides* may be combined.

Dickison, W.C. (1972): Anatomical studies in the Connaraceae. II. Wood anatomy. *J. Elisha Mitchell Sci. Soc.* **88(3):** 120–136.

Veenendaal, G.H., van (1964): Houtanatomie (Connaraceae). *Essay for the Drs degree examination. University of Utrecht, The Netherlands.*

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SEED ANATOMY OF THE ANNONA GROUP (ANNONACEAE)

This report deals with the seed anatomy of the Annona group (Annonaceae). The group consists of five genera: *Annona, Raimondia, Rollinia, Rolliniopsis* and *Anonidium.*

The seed coats are well differentiated and characterized by an outer epidermis, a hypodermis, a mesotesta and an inner epidermis.

The mesotesta is fibrous and in most species consists of two separate layers. The variation within the mesotesta is considerable and five types can be distinguished.

1. Mesotesta composed of an outer layer of longitudinal lignified fibres, and an inner layer of transverse fibres.
2. Mesotesta composed of an outer layer of longitudinal lignified fibres interchanged by areas of transverse fibres, and an inner layer of transverse fibres.
3. Same as type 2, with the exception that the fibres of the outer layer are more interwoven.
4. Mesotesta composed of a transverse outer layer, a longitudinal middle layer and a transverse inner layer.
5. Mesotesta undivided into separate layers.

The seeds of *Annona glabra* are adapted to water dispersal. The endotesta is composed of 6–10 layers of radially piled parenchymatic cells, which function as a buoyancy tissue.

During development, the *Annonaceous* seed becomes ruminate. The ruminations are initiated at the lateral sides of the young seed by local division and elongation of cells in the outer integument. The shape of the ruminations is variable. Five types of ruminations can be distinguished: (1) broad plates, (2) small plates, (3) small plates interchanged with flattened pegs, (4) pegs, (5) clavate.

All the seeds studied exhibit a conical, sclerotic tissue at the micropylar end of the seed. This plug is formed by the exostomal tissue around the micropyle. Its function is to protect the underlying embryo. During germination the plug is pushed out, or to the side of the seed by the root.

The data on seed anatomy indicate that *Rolliniopsis* is closely related to *Rollinia,* whereas *Raimondia* is related to *Annona,* and *Anonidium* is more solitary.

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FUNCTIONAL ANATOMY OF *FICUS ASPERIFOLIA*, A GYNODIOECIOUS TROPICAL FIG

Gynodioecious fig trees have two types of syconia, namely (1) seed figs (functionally female inflorescences that contain long-styled female flowers (seed flowers)), and (2) gall figs (functionally male inflorescences that contain short-styled female flowers (gall flowers) and male flowers). After wasp pollination the seed flowers develop seeds; after wasp pollination and oviposition the gall flowers develop a new generation of wasps. In *F. asperifolia* the gall figs lack a synstigma. The gall flowers are equivalent to the seed flowers in the characters of the embryo sac, but have specialized ovules that facilitate oviposition (ovule dimorphism). In early C phase, both gall and seed flowers show the entrance of a pollen tube into the embryo sac, initial stages of plant embryo development, and endosperm formation. Were it not for the developing wasps, the gall flowers would probably produce seeds. Pollen transport and pollination by wasps provide their offspring with plant endosperm as a nutritive tissue. In the pollinating wasp of the gynodioecious *F. asperifolia,* pollen pockets can be understood as finely adjusted tools for the pollination in oviposited gall flowers and the prevention of seed formation in neighbouring, not oviposited, gall flowers, which would strongly compete for space with the developing galls.